

# Enhanced antitumor efficacy on hepatoma-bearing rats with adriamycin-loaded nanoparticles administered into hepatic artery

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## Abstract

**AIM:** To investigate the antitumor activity of adriamycin (ADR) encapsulated in nanoparticles (NADR) and injected into the hepatic artery of hepatoma-bearing rats.

**METHODS:** NADR was prepared by the interfacial polymerization method. Walker-256 carcinosarcomas were surgically implanted into the left liver lobes of 60 male Wistar rats, which were divided into 4 groups at random (15 rats per group). On the 7th day after implantation, normal saline (NS), free ADR (FADR), NADR, or ADR mixed with unloaded nanoparticles (ADR+NP) was respectively injected via the hepatic artery (i.a.) of rats in different groups. The dose of ADR in each formulation was 2.0 mg/kg body weight and the concentration was 1.0 mg/mL. Survival time, tumor enlargement ratio, and tumor necrosis degree were compared between each group.

**RESULTS:** Compared with the rats that received NS i.a., the rats that received FADR or ADR+NP acquired apparent inhibition on tumor growth, as well as prolonged their life span. Further significant anticancer efficacy was observed in rats that received i.a. administration of NADR. Statistics indicated that NADR brought on a more significant tumor inhibition and more extensive tumor necrosis, as compared to FADR or ADR+NP. The mean tumor enlargement ratio on the 7th day after NADR i.a. was 1.106. The mean tumor-bearing survival time was 39.50 days. Prolonged life span ratio was 109.22% as compared with rats that accepted NS.

**CONCLUSION:** Therapeutic effect of ADR on liver malignancy can be significantly enhanced by its nanoparticle formulation and administration via hepatic artery.

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## INTRODUCTION

Transhepatic arterial chemotherapy (TAC) is recognized as an efficient therapy for liver primary and metastatic tumors by increasing the drug concentration in tumor and improving the therapeutic effect subsequently. It was reported that administration of ADR via the hepatic artery (i.a.) was able to increase the drug concentration in tumor by 3-fold as compared to intravenous administration<sup>[1]</sup>. In patients with cancers, the i.a. administration of ADR reduced the plasma AUC by about 30%<sup>[2]</sup>.

During recent years, nanoparticles have been extensively applied as carriers of antitumor drugs. *In vivo* studies have demonstrated that nanoparticles are specifically concentrated to the reticuloendothelial system (RES), especially to liver and spleen, after administered into body<sup>[3]</sup>. On the basis of these experiences, we hypothesized that a further significant therapeutic effect could be expected by TAC in combination with nanoparticle techniques. We carried out a randomized control study to test it.

## MATERIALS AND METHODS

### NADR preparation and characterization

NADR was prepared by the interfacial polymerization method<sup>[4]</sup>.  $\alpha$ -polybutylcyanoacrylate (PBCA) was used as polymeric materials. The final product had an appearance of reddish, colloidal, semi-transparent solution. Under transmission electron microscopy (TEM), NADR showed a global, regular contour with a homogenous size and distribution. The diameter of particles ranged from 22 to 130 nm (mean, 93.1 nm). The drug-embedding ratio was 82.6%, the drug-loading ratio was 40.9%, and the effusion rate was less than 3% three months later.

### Animals and anesthetic

Sixty male Wistar rats weighing 230-270 g (mean, 250 g) were provided by Laboratory Animal Center of our university and randomly divided into 4 groups, with 15 in each. Sumianxin (Changchun Agricultural Pastoral University) was used as anesthetic.

### Establishment of hepatoma model

One mL of suspension containing 10<sup>7</sup> Walker-256 (W256) carcinosarcoma cells (Shanghai Medical Industrial Institution) was injected into the thigh muscle of a carrier rat (not included in experimental rats). One week after inoculation, a palpable tumor generated at the injected site. Viable tumor tissue was excised under sterile conditions and soaked in 20 mL of Hanks balanced salt solution. Tissue was diced into approximately 1 mm×1 mm×1 mm fragments. Experimental rats were anesthetized with intramuscular injection of Sumianxin at 0.2 mL/kg. Median incision beneath the metasternum was made and the liver was exposed. The tumor fragment was implanted into the left liver lobe.

### Administration i.a.

On the 7th day after tumor implantation, all animals received laparotomy again. The longest (a) and shortest (b) diameters of

the tumor were measured. The tumor volume was calculated as

$$\frac{a \times b^2}{2}$$

By cannulation method described previously<sup>[5]</sup>, normal saline (NS), free ADR (FADR), NADR, or ADR mixed with unloaded nanoparticles (ADR+NP) was injected into the hepatic artery of rats in groups 1-4 respectively. The dose of ADR in each formulation was 2.0 mg/kg body weight. The concentration of ADR was 1.0 mg/mL.

### Assessment of therapeutic effect

**Tumor growth inhibition** Seven days later, all animals received the third laparotomy. The longest (a) and shortest (b) diameters of the tumor were measured again and the tumor volume after administration was calculated. The tumor volume ratio (TVR) was calculated as

$$\text{TVR} = \frac{\text{Tumor volume after administration}}{\text{Tumor volume before administration}}$$

**Tumor necrosis degree** Seven random rats in each group were killed and anatomized. Hepatoma was removed completely and fixed in 40 g/L formaldehyde. Three 5-μm thick sections in each tumor were cut on the maximal transverse plane and mounted on glass slides overnight at room temperature. After HE staining, the sections were examined under microscope. According to the percentage of necrosis area, tumors were graded on the following criteria: I, 0-30%; II, 30-70%; III, 70-100%.

**Increased life span** Survival time of the remaining 8 rats in each group was recorded. The mean survival time of NS group was reckoned as control. Increased life span (%ILS) was calculated as

$$\% \text{ILS} = \left( \frac{\text{Mean survival of treated group}}{\text{Mean survival of control group}} - 1 \right) \times 100\%$$

### Statistical analysis

SPSS 10.0 for windows was used for statistical analysis. Statistical significance was tested by ANOVA and Dunnett test for tumor growth inhibition, log rank test for survival time.  $P < 0.05$  was considered statistically significant.

## RESULTS

### Tumor growth inhibition

No difference in tumor volume was found among groups before treatment ( $P > 0.05$ , Table 1). After treatment, the tumor grew rapidly in rats that received NS. The mean tumor volume was 31.55 times greater than that before treatment. Metastases were observed in about half of these rats. In contrast, the speed of tumor growth lowered apparently in rats that received FADR or ADR+NP ( $P < 0.01$ ). No difference between FADR and ADR+NP was observed ( $P > 0.05$ ). The slowest tumor growth was found in rats that received i.a. administration of NADR. Statistics indicated that NADR brought on a further significant tumor inhibition, as compared with FADR or ADR+NP ( $P < 0.01$ ). In addition, no metastasis was found in rats that received NADR.

### Tumor necrosis degree

Under microscope, W256 tumor cells in rats that received NS appeared active proliferation and extensive mitoses. Small areas of necroses were observed in the center of tumor tissue and accompanied with a few inflammatory cells (Table 2). Moderate to severe necroses were found in tumors of rats that

received FADR or ADR+NP. Grade III of tumor necroses was found in 5 of 7 tumors after administration of NADR, including 2 cases of complete tumor necrosis.

### Increased life span

Compared with the survival time (18.88 d) in saline controls, the tumor-bearing survival time was greatly prolonged in animals that received NADR (mean, 39.50 d), or FADR (mean, 27.75 d), or ADR+NP (mean, 26.13 d) (Table 3). NADR prolonged the life span by 109.22%, which was longer than FADR (46.98%) and ADR+NP (38.40%) ( $P < 0.05$ ).

**Table 1** Tumor volume (V) and tumor volume ratio (TVR) after treatment (mean±SD)

Group	V (cm <sup>3</sup> )		TVR
	Before treatment	After treatment	
NS	0.086±0.049	2.521±0.840	31.550±7.975
FADR	0.083±0.035	0.149±0.072	1.883±0.708 <sup>b</sup>
ADR+NP	0.079±0.036	0.161±0.105	1.896±0.565 <sup>b</sup>
NADR	0.079±0.033	0.087±0.038	1.106±0.275 <sup>d</sup>

<sup>b</sup> $P < 0.01$ , vs NS; <sup>d</sup> $P < 0.01$ , vs FADR, ADR+NP.

**Table 2** Tumor necrosis degree after treatment

Grade	NS	FADR	ADR+NP	NADR
I	6	2	1	0
II	1	3	5	2
III	0	2	1	5 <sup>1</sup>

<sup>1</sup>Complete necrosis in 2 cases.

**Table 3** Mean survival time and increased life span (%ILS) after treatment (mean±SD)

Group	Mean survival time (d)	%ILS
NS	18.88±2.80	-
FADR	27.75±6.34	46.98 <sup>b</sup>
ADR+NP	26.13±5.75	38.40 <sup>b</sup>
NADR	39.50±8.97	109.22 <sup>a</sup>

<sup>a</sup> $P < 0.05$ , vs FADR, ADR+NP; <sup>b</sup> $P < 0.01$ , vs NS.

## DISCUSSION

We did not include intravenous administration (i.v.) in the present experiment because the i.a. route was much more efficient than i.v. in the treatment of liver malignancies. Different from the liver parenchyma, which receives more than 70% of its blood supply from the portal vein and the rest from the hepatic artery, hepatoma receives approximately 90% of its blood supply from the hepatic artery. The speciality of liver blood supply determines the great difference between the two routes of administration. The difference did not warrant repeat comparison in our study.

The results of our experiments demonstrated that the antitumor activity of ADR could be markedly enhanced when the drug was encapsulated in nanoparticles and administered into the hepatic artery. Equivalent or similar effects could not be acquired using FADR or ADR+NP. Compared with FADR or ADR+NP, NADR produced a more significant tumor inhibition and more extensive tumor necrosis. The average tumor volume on the 7th day after treated with NADR was 0.087 cm<sup>3</sup>, nearly the half of 0.161 cm<sup>3</sup> of the volume after treated with ADR+NP. The survival time of rats that received NADR was evidently increased. Compared with saline

controls, NADR prolonged the life span by 109.22%.

The likely explanations for the improved therapeutic activity in four aspects are as follows. First, it has been testified that the cytotoxic effect of ADR depends on the concentration and duration of exposure<sup>[6]</sup>. Increasing ADR concentration in tumor cells or slowing its elimination could certainly enhance its antitumor efficacy. Secondly, encapsulating the drug into nanoparticles might modify its distribution pattern in tissues. After administered into body, nanoparticles were taken up selectively by RES, such as the liver, spleen, and bone marrow. Accumulation of biodegradable nanoparticles with associated drugs in Kupffer cells would create a gradient of drug concentration for a massive and prolonged diffusion of the free drug towards neoplastic tissues<sup>[3]</sup>. Thirdly, *in vitro* studies reported that compared to FADR, NADR exhibited a 3-fold enhancement of cytotoxicity, as determined by cell growth inhibition and DNA synthesis, after continuous exposure to 0.02 and 0.04 µg/mL<sup>[7]</sup>. Further studies showed that nanoparticulate pharmaceuticals were able to enter specifically certain types of tumor tissues or tumor cells<sup>[8]</sup>. Fourthly, the tiny size of nanoparticle could increase its contact areas significantly, which would result in an apparent improvement in its bioavailability. A recent study compared carbendazim, a novel anticancer drug, with its nanoparticles. The result confirmed that nanoparticle formulation improved the drug bioavailability by 166%<sup>[9]</sup>.

Our experiments support the hypothesis that the therapeutic effect could be dramatically enhanced by TAC in combination with nanotechnology. We conclude that nanoparticles can be used as a promising drug carrier in TAC for the treatment of liver primary and metastatic tumors.

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